Functional Autonomy of Monopolar Spindle and Evidence for Oscillatory Movement in Mitosis

ANDREW S. BAJER
Department of Biology, University of Oregon, Eugene, Oregon 97403

ABSTRACT The oscillations of chromosomes associated with a single spindle pole in monocentric and bipolar spindles were analysed by time-lapse cinematography in mitosis of primary cultures of lung epithelium from the newt *Taricha granulosa.* Chromosomes oscillate toward and away from the pole in all stages of mitosis including anaphase. The duration, velocity, and amplitude of such oscillations are the same in all stages of mitosis. The movement away from the pole in monocentric spindle is rapid enough to suggest the existence of a previously unrecognized active component in chromosome movement, presumably resulting from a pushing action of the kinetochore fiber. During prometaphase oscillations, chromosomes may approach the pole even more closely than at the end of anaphase. Together, these observations demonstrate that a monopolar spindle is sufficient to generate the forces for chromosome transport, both toward and away from the pole.

The coordination of the aster/centrosome migration in prophase with the development of the kinetochore fibers determines the course of mitosis. After the breaking of the nuclear envelope in normal mitosis, aster/centrosome separation is normally followed by the rapid formation of bipolar chromosomal fibers. There are two aberrant extremes that may result from a failure in coordination between these processes: (*a*) A monocentric spindle will arise when aster separation does not occur, and (*b*) an anaphaselike prometaphase will result if the aster/centrosomal complexes are already well-separated and bipolar chromosomal fibers do not form. In the latter case, the two monopolar prometaphase half-spindles migrate apart, each containing a random number of two chromatid (metaphase) monopolar-oriented chromosomes. This random segregation of prometaphase chromosomes displays many features of a standard anaphase and may be followed by a false cleavage. The process of polar separation during prometaphase occurs without any visible interzonal structures. Aster/centrosomes and monopolar spindles migrate autonomously by an unknown mechanism. There are, however, firm but transitory connections between the aster center and the kinetochores as demonstrated by the occasional synchrony of centrosome-kinetochore movement. The data suggest that aster motility is important in the progress of both prometaphase and anaphase in normal mitosis.

The bipolar mitotic apparatus normally provides the structural basis for equal chromosome segregation. The observations reported here demonstrate, however, that a bipolar spindle is not necessary either for chromosome-to-pole movement or for polar migration. Thus, a monopolar spindle or "half-spindle" appears to be the basic functional unit of mitosis.

In a standard bipolar mitotic spindle, two essential steps assure precise segregation of the genetic material. In the first, double-chromatid chromosomes are bipolar oriented; in the second, they are aligned in an equatorial, metaphase position. Thus, the sister kinetochores come to point toward two opposite poles. After chromosome splitting in the kinetochore region at the start of anaphase, sister chromosomes migrate in opposite directions. Failure to follow these steps may lead to malfunctions in the process of mitotic segregation. For example, persistent monopolar chromosomal orientation and/or the failure to form a bipolar spindle results in monocentric division. The random premature distribution of chromosomes in prometaphase, when they still have double-chromatid structure, leads to anaphaselike prometaphase (9).

Monocentric divisions, occurring either naturally or as the result of experimental manipulation, have been described in

THE JOURNAL OF CELL BIOLOGY · VOLUME 93 APRIL 1982 33-48

various groups of organisms. Fankhauser (19) studied monocentric spindles after polyspermic fertilization in the newt. Metz (34, 35) observed them during spermatogenesis of the fly *Sciara*, Scott (54) identified them in paedogenetic reproduction in the beetle *Micromalthus*, and Fux (20) and Camenzind and Fux (17) described them in the gall midge *Mycophila*. The monocentric spindle is a common result of a variety of experimental treatments such as low temperature (43) or colchicine (11), especially in tissue culture cells (18) and in the eggs of marine animals (31, 33, 40, 57).

Anaphaselike prometaphase, unlike monocentric division, is a little-known phenomenon, although it was reported in cancer cells by Galeotti in 1893 (cited in reference 60). Peters (Fig. 18 of reference 41) shows a typical anaphaselike prometaphase chromosome arrangement in cornea cells of *Triturus* recovering from colchicine treatment but makes a few comments concerning this configuration; Zirkle (p. 536 of reference 64) mentions six (not analyzed) time-lapse records of such division in newt heart fibroblasts. The chromosome distribution, the structure of asters, and the false interzone that occur during this process have been studied in a tetraploid line of PtK_2 cells where the process was compared to a random first meiotic division (15). In the present study, certain aspects of monocentric division and anaphaselike prometaphase are analyzed to promote an understanding both of the origin of these two phenomena in diploid newt cells and of the conditions required for transformation of a monocentric spindle into a bipolar configuration. It appears that the major factor in these malfunctions is an unexplained tendency towards the long-distance migration (7, 9) of aster/centrosomal complexes. Fig. 1 summarizes the observed relations between the malfunctions that may occur spontaneously in untreated cells in tissue culture.

Chromosome oscillations in anaphase, although previously observed (14), have drawn little attention in the literature (45). This report is the first in which the oscillations of monopolar oriented chromosomes in monocentric division are analyzed in detail and compared to similar oscillations that occur in standard bipolar anaphase. The pattern of chromosome migrations in prometaphase monopolar spindles and the chromosome oscillations that occur during anaphase are very similar. This suggests that chromosome migration in all stages of mitosis may not be unidirectional but may occur in an oscillatory fashion. The mitotic mechanism remains unknown, but it may involve an "oscillating" system capable of transporting material in two opposite directions within the same spatial domain in quick succession. This feature of mitosis in the newt may turn out to be a significant aspect of a general mitotic mechanism.

The present analysis provides convincing evidence both of pushing by chromosomal and/or astral fibers in living cells and of lateral interactions between neighboring chromosome fibers. These observations on the migration of monocentric spindle lead to reevaluation of the concept of the central spindle (32). The central spindle present in numerous lower organisms (21, 26, 58) may not have a homologous counterpart in the astral mitosis of higher organisms.

Terminology

Some of the phenomenon described here are known in the literature by different names, or else the published nomenclature is not used consistently. Therefore, the less familiar terms and those with subtle distinctions are discussed below.

ANAPHASELIKE PROMETAPHASE VS. FALSE ANAPHASE: Anaphaselike prometaphase (9) is the random, usually transitory segregation of a diploid or multiploid chromosome set shortly after the breaking of the nuclear envelope. It is not preceded by formation of the metaphase plate. Chromosomes still contain two chromatids, not single chromatids as in normal anaphase. The region between separating double-chromatid chromosome groups is called "false interzone." Anaphaselike prometaphase has been called a meiosislike division or "false anaphase" by Brenner et al. (p. 374 of reference 15).

"False anaphase" as defined by Zirkle (64) is, however, a different type of mitotic disturbance. It occurs after a prolonged metaphase with what is probably a very short, bipolar spindle. The metaphase chromosomes are distributed randomly, often as "rosettes" in star configurations. False anaphase is the terminal stage of an aberrant mitosis; it also has been observed in newt epithelial cultures (A. S. Bajer et al., unpublished observations). Anaphaselike prometaphase is not a final stage

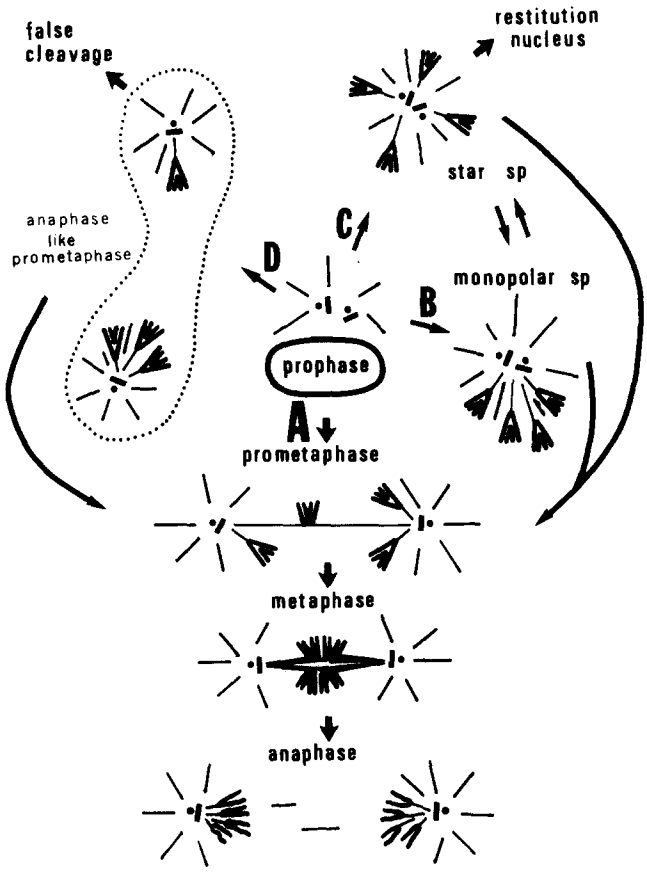


FIGURE 1 Migration of centrosomes with asters and development of the spindle. Arrows show possible modifications and transformations of the spindle during the course of mitosis in the newt epithelium. *A*, Standard course of mitosis. Extreme cases are monopolar spindle (*B*), star configuration (*C*), and anaphaselike prometaphase (*D*). *B–D* result from either the delay of centrosome separation or the dissociation from the spindle of one aster without any chromosome. Anaphaselike prometaphase results from the failure of formation of bipolar kinetochore fibers and precocious migration of asters. These malfunctions tend to be corrected during a considerably prolonged (long arrows) prometaphase, which is not always fully successful and may result in the formation of a multipolar spindle. Thus, prometaphase-metaphase is a prerequisite for bipolar (multipolar) chromosome distribution. If these malfunctions are not corrected, then false cleavage occurs (cytokinesis divides prometaphase chromosomes into two sets of arbitrary size) or a restitution nucleus (4n) is formed.

of mitosis and formation of a metaphase plate and a standard anaphase may follow.

MONOCENTRIC VS. MONOPOLAR SPINDLES: A monocentric spindle is a structure, not necessarily spindle-shaped, in which the chromosomes are functionally connected to a single pole. This term refers either to a monopolar spindle, which is a single cone and not a spindle-shaped structure, or to a star configuration. In a star configuration, the chromosomes are arranged in a circle (in flat cells) or a sphere (in round ones). Star configurations correspond to certain types of monasters, a term not used here. A monocentric spindle leads to an abortive, monocentric mitosis and a 4n restitution nucleus.

MONOPOLAR VS. BIPOLAR ORIENTATION AND CENTROPHILIC CHROMOSOMES: Mitotic chromosomes that are functionally connected to a single pole are said to be monopolar oriented; those connected to two poles are called bipolar oriented. In the prometaphase bipolar spindle the term centrophilic (59, 63) refers to chromosomes that associate with a single pole rather than migrating to the spindle equator. The term will be used here interchangeably with monopolar-oriented chromosomes, because centrophilic chromosomes have either one or two sister kinetochores connected to a single pole (38) until reorientation. In the former case, one of the kinetochores is lacking kinetochore fiber (9; J. Molè-Bajer and A. S. Bajer, manuscript submitted for publication).

MATERIALS AND METHODS

Primary cultures of the lung epithelium of the Oregon newt (*Taricha granulosa, granulosa*, with 2n = 22) were used. Monolayer primary cultures were grown in modified (49) Rose chambers (47) in Liebowitz' (28) L-15 medium at pH 7.25. The methods were described previously (9, 45). Observations were made at 21°–22°C, although no differences were found in cultures grown at 26°C. Table I gives the typical distribution of the mitotic stages and disturbances studied in these cultures.

Time-lapse recording was done with a phase (32× Leitz) objective, N.A. 0.40, combined with a Reichert anoptral condenser and Wild photographic eyepieces (5×, 6×, and 8×). Numerous cells were also recorded with DIC Nomarski optics (Zeiss) and a polarizing microscope with Nikon rectified optics. Both these systems, however, require disassembly of the Rose chambers which often results in the cells rounding up. Most of the records analyzed are of cells photographed with phase contrast in Rose chambers in their undisturbed growth environment. Recording was done on Kodak 16-mm negative film Technical Pan 2415 (AH Estar base), processed in Kodak developer HC-110. All films were taken with an Arriflex 16-mm camera equipped with a register pin, assuring the precise alignment of consecutive frames. L-W projectors (Mark V and Athena 224-B; L-W International, Woodland Hills, CA) were used either alone or in conjunction with a chart recorder for film analysis. Projection at 1 frame/s onto a chart recorder with paper transport (12.5 cm/min) perpendicular to the direction of migration permitted one to follow the movement of superimposed chromosomes which would have been more difficult with standard frame-by-frame analysis. Allowances were made for changes of directions by rotating the chart recorder. Usually, the movement of only a few chromosomes in one cell could be analyzed, but in some cells, over 20 chromosomes could be followed. As the centrosome is not visible in all cells during the whole process of mitosis, its position was often estimated with a precision of ~1 μm as the center toward which the chromosomes oscillate, i.e., as the point where the extrapolated paths of the chromosome oscillations intersect (following Wolf [61, 62]). The measurements of the paths of oscillating chromosomes were done either by frame-by-frame analysis or directly during slow projection with a Panasonic electronic ruler, JE 8210 U. The latter type of measurements contain an ~20% error.

The present analysis revealed that slow projection rates may result in considerable distortion of movements due to the mechanical design of most projectors. This presents additional difficulties for the analysis of movements. The LW projectors used in the present analysis have reasonable vertical registration, but the lateral alignment of successive frames is poor. This affects the precision of the measurements, depending on the orientation of the object in the frame. Therefore, only well-defined displacements in space (1 μm and longer) will be discussed here.

RESULTS

Bipolar vs. Monopolar Orientation

Bipolar chromosome orientation, which is a prerequisite for equal chromosome distribution, is achieved in prometaphase and finalized in metaphase.

A delay in the formation of bipolar kinetochore fibers and consequent monopolar orientation is a recurrent feature of urodele and PtK_1 epithelial cells (46) and probably of astral mitosis in general. Monopolar chromosomes in the newt migrate towards the centrosome where they tend to be radially arranged until reorientation.

Typical bipolar and monopolar orientations of double chromatid chromosomes can often be distinguished in living cells (Fig. 2). The arms of monopolar oriented chromosomes are rather J or U shaped. Their kinetochore regions point toward a single pole and usually move without change of shape. The significance of rarely observed changes of shape is discussed below. In contrast, the distal parts of bipolar oriented chromosomes are often nearly motionless; only the kinetochores and the proximal regions of the arms oscillate.

Aster Mobility and Prometaphase Elongation of the Spindle

Newt mitosis is typified by large asters best visualized by immunochemical techniques (9). The two centrosome/aster complexes that start migration in prophase are separated by varying distances during the breaking of the nuclear envelope. Immunochemical studies did not detect any traces of a central spindle between separating chromosomes. As such, they are in agreement with EM data on corresponding newt (36) and rat kangaroo (44) cells. The centrosome is often visible in the center of the aster, even in phase microscopy (Fig. 2). It has a diameter of ~0.4 μm. Its displacement is occasionally detected on consecutive frames of 16-mm film, e.g., at 6- to 10-s intervals. It can move in diverse ways, ranging from a straight line to an erratic rocking.

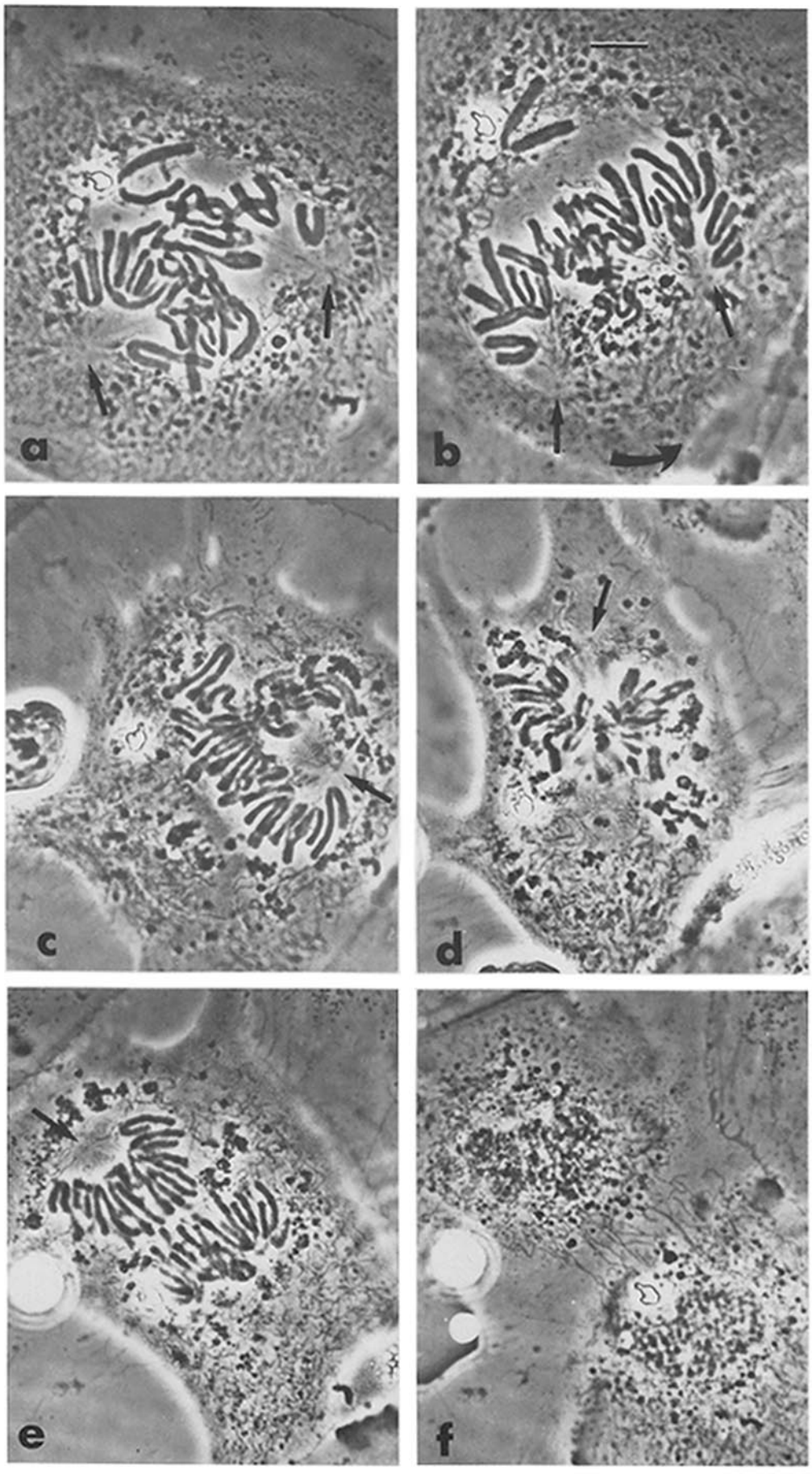
The course of early prometaphase depends to a great extent on the distance between the kinetochores and the centrosomes/asters as well as on the orientation of kinetochores at the moment the nuclear envelope breaks. These factors influence the appearance of functional kinetochore fibers during early

TABLE I

Mitosis in Primary Cultures of Newt Lung Epithelium: Distribution of Mitotic Stages and Mitotic Aberrations in Primary Explants: 21°C

No. of explants	Prophase	Prometaphase	Metaphase	Anaphase	Telophase	Anaphase-like prometaphase	Monopolar spindle	Star
34	51	43	24	7	34	3	22	29

The number of prometaphases should be >43, since a high percentage of the monopolar-star configurations transforms eventually into a standard prometaphase. The number of monopolar-star configurations was exceptionally high in these cultures. 28 of 43 prometaphases had centrophilic chromosomes, and in four cells (three monopolar and one prophase) single asters without chromosomes were observed in different stages of dissociation (see reference 9).


a
b
c
d
e
f

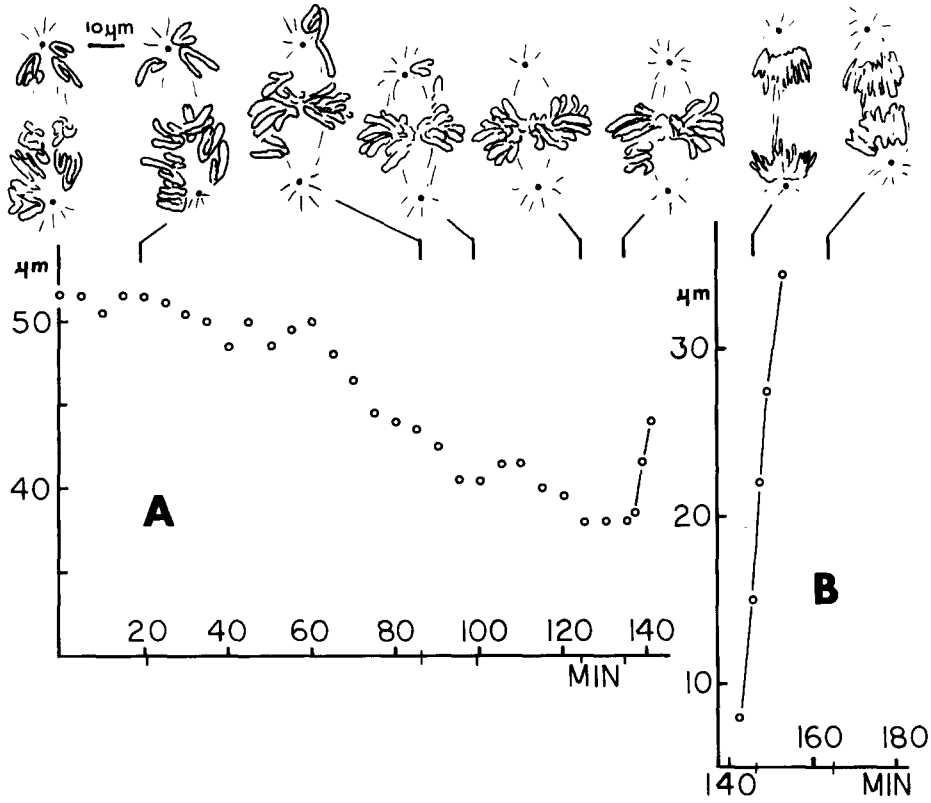


FIGURE 3 The shortening of a prometaphase spindle. The distance between centrosomes (*A*) and between sister chromosome groups (*B*) plotted against the time. The comparatively slow (over 2 h) shortening of the spindle occurs at variable rates and is interrupted by periods of elongation (60 min and 110 min). The highest rate of shortening is ~0.7 μm/min, while for comparison each sister chromosome group in anaphase of this cell moves nearly twice as fast (1.25 μm/min). Anaphase starts at 138 min. Cell 96/80. 21°C.

prometaphase; such fibers are detected especially well in polarizing microscopes (8, 50). The elongating, early prometaphase spindle is very thin and may contain as few as two bipolar oriented chromosomes; some centrophilics are often located close to both poles. In cultured newt epithelial cells, as in meiosis of crane fly (27), the spindle is shortest at the end of metaphase. The shortening and thickening of the newt prometaphase spindle occurs during the reorientation of the monopolar chromosomes located at both poles and their migration to the equatorial plate (Fig. 3). If a metaphase plate is established, a standard anaphase follows. Prometaphase is, however, the most unpredictable stage of mitosis showing two aberrant extremes: the first is anaphaselike prometaphase and the second, monocentric division. These extreme malfunctions result from improper timing of three normal processes: aster migration (centrosome separation), the breaking of the nuclear envelope, and the formation of bipolar kinetochore fibers.

Anaphaselike Prometaphase

Certain cells contain normally separating asters with centrosomes, but the formation of bipolar kinetochore fibers after breaking of the nuclear envelope is delayed for ~10 min (at 21°–22°C). In these cases all the double-chromatid chromosomes become randomly arranged in monopolar orientation toward the two poles (asters). The two prometaphase half-spindles, each with a random number of chromosomes, continue their migration in an anaphaselike fashion. The breaking of the nuclear envelope is, therefore, not followed by formation of a bipolar spindle (Figs. 4 and 5) as in standard prometaphase. The distance of migration toward the cell periphery varies from ~30 μm (which is the approximate length of the metaphase spindle) to ~150 μm, i.e., about four times longer than anaphase separation. During the migration of these prometaphase half-spindles, chromosomes oscillate in the same way as in monocentric spindles with a diploid set (see next section, "Monocentric Spindle"). This process is rather dramatic during film projection: one or both asters with monopolar-oriented chromosomes migrate toward the periphery of the cell; very little, if any, shortening of the chromosomes-to-pole distance is seen. The cell may finally pinch into two (seven cases with three time-lapse records).

In most cases, one of the monopolar spindles, often containing only a few chromosomes, rotates at the cell periphery 90°–180° (Figs. 4 and 5). This provides additional evidence that the interzone of the type found in standard anaphase is not required in the separation of the poles. The rotation of the aster is followed by its migration toward the other, often stationary aster/centrosome. Migration, often taking place in an erratic manner, finally leads to the fusion of the two prometaphase half-spindles and the formation of a bipolar or multipolar spindle. "Backward" migration without previous rotation has not been observed. The fusion of two prometaphase half-spindles always occurs in a similar manner, i.e., nearly sideways and not back-to-back. The fusion begins after

FIGURE 2 Aster behavior and monopolar vs. bipolar orientation. Due to the pronounced mobility of the asters, the whole mitotic apparatus rotates. Centrosomes (marked by arrows in *a–e*) are not visible in all frames, because of their movements out of focus and the change of cell shape. Monopolar oriented (centrophilic) chromosomes close to the poles, seen in *a–c*, resemble anaphase orientation. Only about one third of prometaphase chromosomes (at *a*) are initially bipolar oriented, and they form a thin bipolar spindle. During rotation of the mitotic apparatus (curved arrow in *b*) asters have a tendency to dissociate from the spindle, and the mitotic apparatus assumes a somewhat crescent shape. Normal metaphase (*d*), anaphase (*e*), and cleavage follow. Time in minutes after *a*: *b*, 60; *c*, 97; *d*, 245; *e*, 268, *f*, 323. 21°C. Bar in *b*, 10 μm.

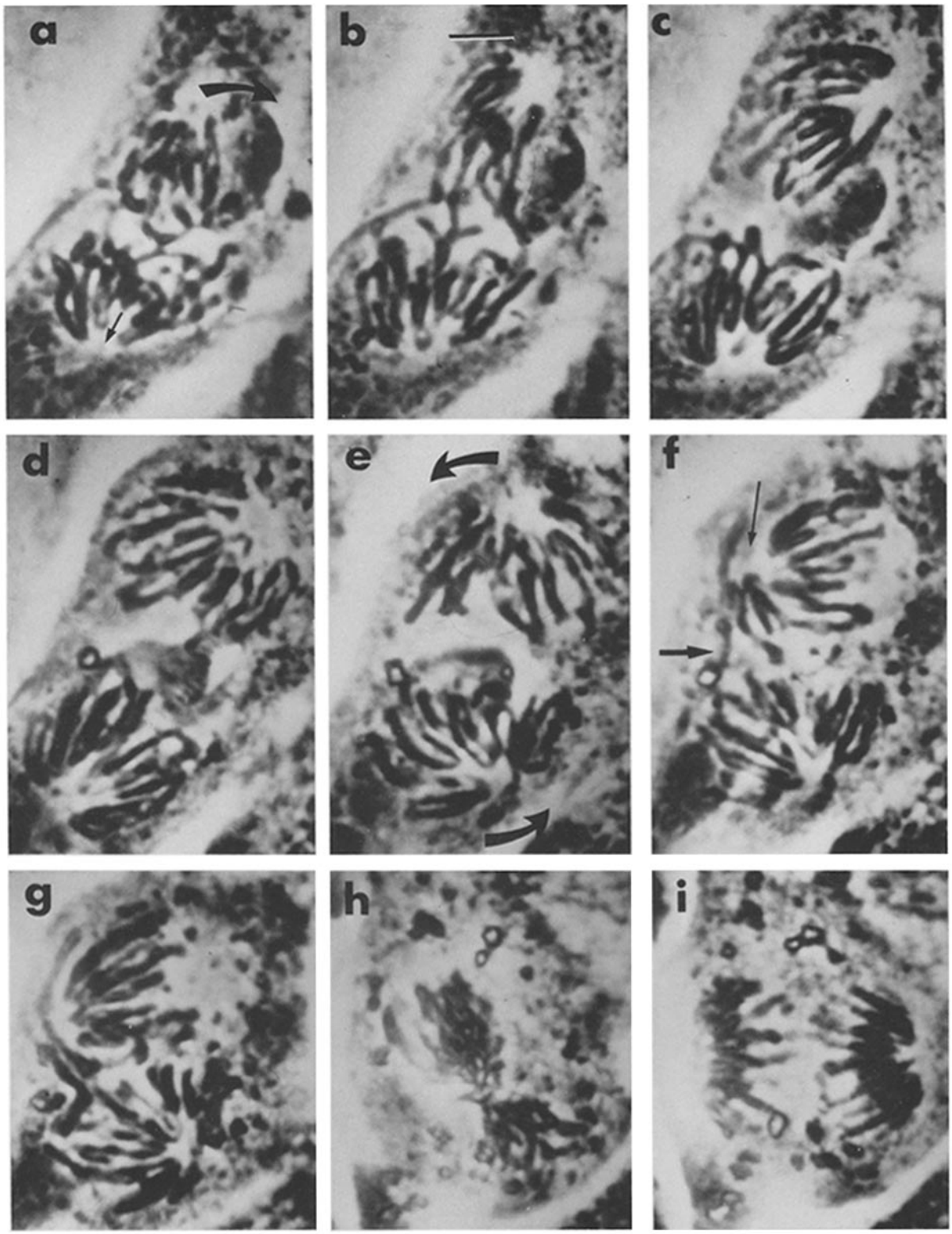


FIGURE 4 Anaphaselike prometaphase followed by formation of a bipolar spindle. Centrosomes are only seen in some frames (thin arrow in *a* and *f*). The lower half-spindle is comparatively stationary while the upper one migrates and rotates to the right (curved arrow at *a*). This is followed by a migration up and finally to the left (curved arrow in *e*). At the same time (*e*) the lower half-spindle rotates to the right and a few chromosomes become bipolar oriented (arrow in *f*). Their number increases and a standard metaphase is followed by anaphase and cleavage (not shown). The process of fusion lasts ~1 h. The duration of anaphase is normal as seen from *h* (beginning of anaphase) and *i*. (See also Fig. 5, which shows the same process in a different cell.) Time in minutes after *a*: *b*, 6; *c*, 14; *d*, 31; *e*, 50; *f*, 58; *g*, 63; *h*, 123; *i*, 135. 22°C. Bar in *b*, 10 μm.

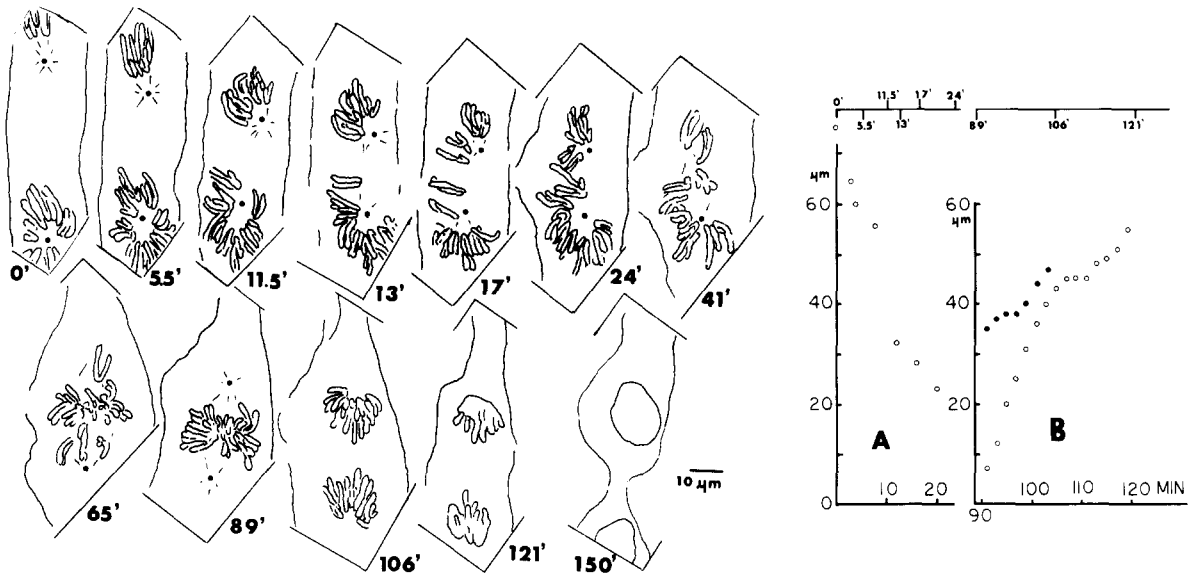


FIGURE 5 Anaphaselike prometaphase and fusion of prometaphase half-spindles. The time in minutes is marked on drawings. (*A*) The distance between centrosomes and (*B*) sister chromosome groups (white circles) and centrosomes (black circles). Recording of this cell started when one (upper) chromosome group began its return movement toward the other, nearly stationary prometaphase half-spindle. The upper half-spindle was a typical monopolar spindle and the lower a star configuration. Chromosome arrangement in the star configuration began to change when the centrosomes were ~55 μm apart, i.e., nearly twice as long as the average metaphase spindle (30 μm). The chromosomes from both chromosome groups began to migrate toward the center (equator) at 11.5 min (*11.5'* on figure represents 11.5 min). These chromosomes formed a thin bipolar spindle (13–24 min) and the others gradually migrated one by one to the metaphase plate. Velocity of anaphase separation between the two chromosome groups (2.7 μm/min) was somewhat slower than the return migration during anaphaselike prometaphase (3 μm/min) which, however, was rather rapid in this cell. Cell 121/80. 22°C.

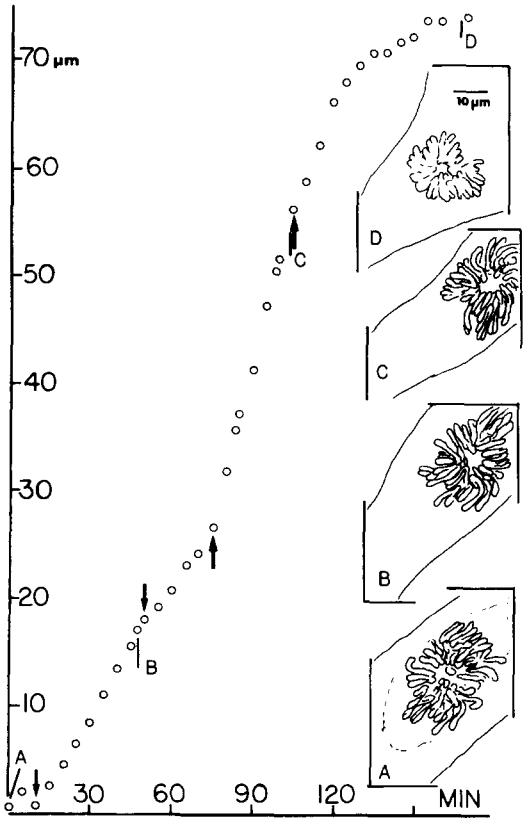


FIGURE 6 Migration of star configuration. The distance of the center of star from an arbitrary point plotted against time. The maximum velocity (1 μm/min) of this star is somewhat slower but within the range of anaphase migration of a single sister chromosome group. (Insets *A–D*) Drawings of the star configuration in positions *A–D* at left. Note that in *D* the formation of the restitution nucleus has already started. The movements between two arrows pointing downward and upward are at constant velocity. Cf. also Fig. 5. Cell 19/80. 21°C.

one or a few chromosomes establish bipolarity (Fig. 5); then the remaining chromosomes behave like centrophilics during a standard prometaphase, gradually becoming bipolar oriented and migrating toward the equatorial region.

Anaphaselike prometaphase in our newt cultures is rare, with an estimated frequency of 1:100 ± 50. The very start of this process, which begins shortly after breaking of the nuclear envelope, was observed only once. In all other cases, anaphaselike prometaphase has been noticed only when chromosome groups were already separated (cf. also reference 15). Anaphaselike prometaphase has been observed in 31 cells including cells prepared for immunochemical (9) and EM studies. The following chromosome distributions were observed in eight cells: 3–19 (two times), 4–18, 6–16, 7–15, 8–14, 10–12, and 11–11. Because of the chromosome configuration in other cells, a precise count was impossible but was estimated to be between 8–14 and 11–11. In 18 time-lapse records, anaphaselike prometaphase was followed either by normal metaphase and anaphase (14 cells) or by multipolar anaphase (4 cells). In several other cells, however, the migration of asters with centrosomes ceased simultaneously with the appearance of bipolar-oriented chromosomes. These cases may be considered as a transition from anaphaselike prometaphase to normal prometaphase.

Monocentric Spindle

The failure of astral separation or the dissociation of one aster from the spindle in prophase (9, 19, 55) results, as a rule,

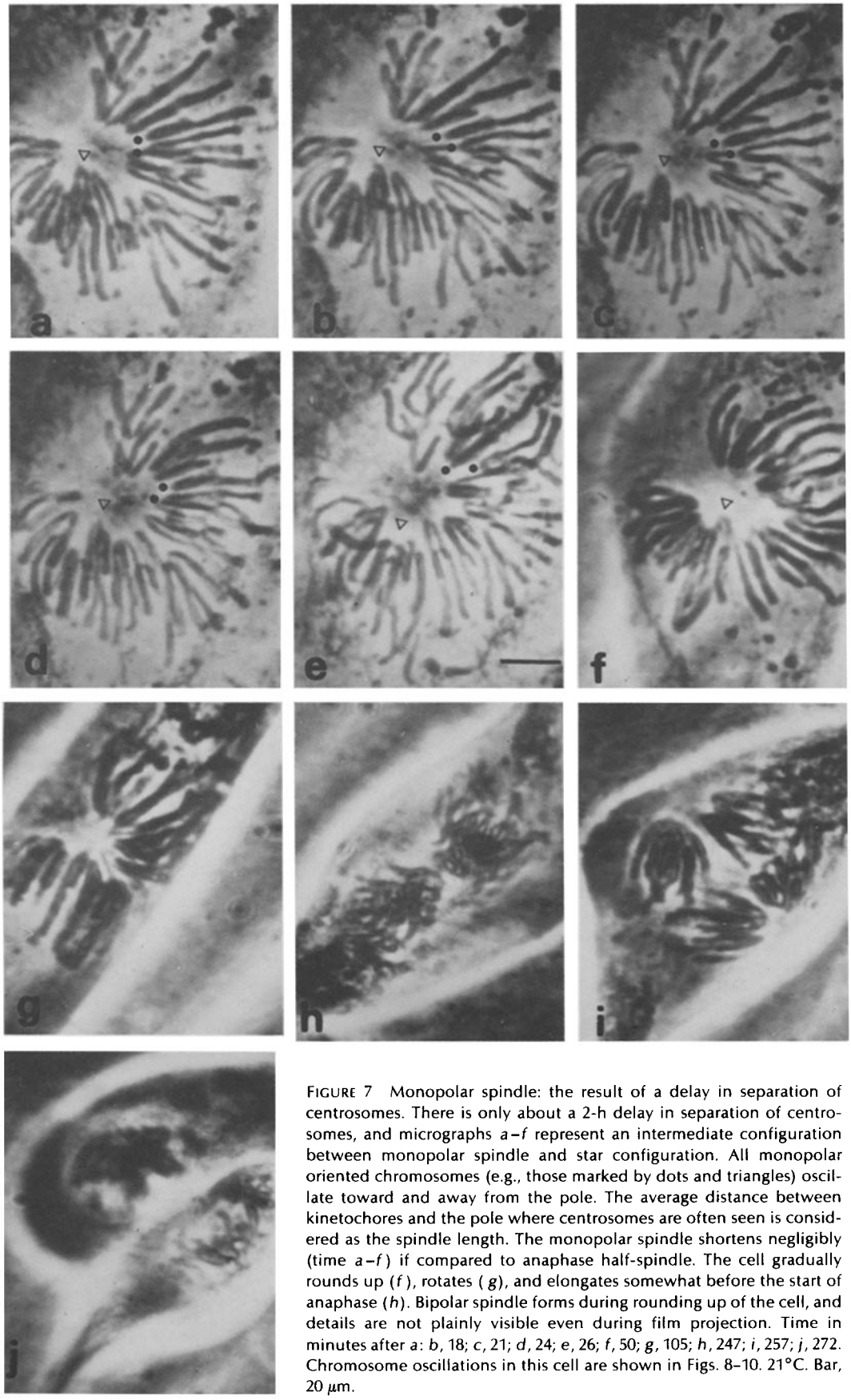


FIGURE 7 Monopolar spindle: the result of a delay in separation of centrosomes. There is only about a 2-h delay in separation of centrosomes, and micrographs *a*–*f* represent an intermediate configuration between monopolar spindle and star configuration. All monopolar oriented chromosomes (e.g., those marked by dots and triangles) oscillate toward and away from the pole. The average distance between kinetochores and the pole where centrosomes are often seen is considered as the spindle length. The monopolar spindle shortens negligibly (time *a*–*f*) if compared to anaphase half-spindle. The cell gradually rounds up (*f*), rotates (*g*), and elongates somewhat before the start of anaphase (*h*). Bipolar spindle forms during rounding up of the cell, and details are not plainly visible even during film projection. Time in minutes after *a*: *b*, 18; *c*, 21; *d*, 24; *e*, 26; *f*, 50; *g*, 105; *h*, 247; *i*, 257; *j*, 272. Chromosome oscillations in this cell are shown in Figs. 8–10. 21°C. Bar, 20 μm.

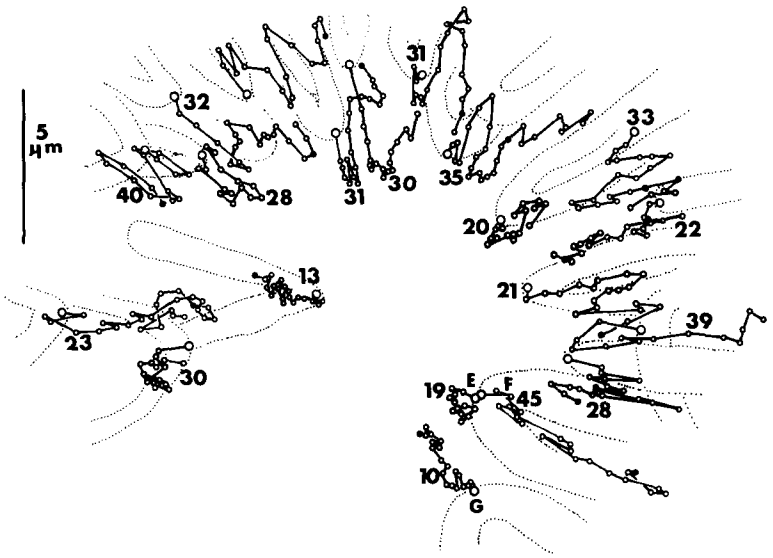


FIGURE 8 Kinetochore path during 10 min of chromosome oscillations in a monopolar spindle. The numbers show the distance (μm) traveled in 20 min. This spindle transforms into a standard bipolar one (Fig. 7). Oscillations of some chromosomes are shown in Fig. 9. Large circles mark the beginning (0 time) of measurements and small black ones mark 10 min later. Measurements (white circles) were done every 30 s. The paths of several chromosomes are superimposed and, for clarity, longer paths are drawn only for a few chromosomes, and the paths of all chromosomes are not shown. After 10 min of migration, the location of some kinetochores is close to their starting position while others migrate ~7 μm (cf. *E*, *G*, and *F*). Kinetochores in the same general region shown (e.g., upper part of the spindle and right side) have similar paths, with some exceptions. Chromosomes at the edge usually oscillate less (13, 10). Few chromosomes usually located farther from the center make long migrations (37, 43; J. Molè-Bajer and A. S. Bajer, manuscript submitted for publication). There is a general slow clockwise displacement of several chromosomes in the upper part of the spindle which varies for different chromosomes. The paths are either straight for a distance of a few μm or oscillate somewhat sideways, especially when chromosomes do not migrate for a long distance. Cell 88/80. 21°C.

in the formation of a monopolar spindle. Chromosomes tend to swing around the pole, which leads to slow transformations into a star configuration (Fig. 1). This occurs without a change in the average distance between the kinetochores and the centrosome. The transformation usually lasts a few hours at 22°C; the kinetochores (chromosomes) finally become arranged in a complete, or nearly complete, circle in flat cells, or at the periphery of a sphere in round ones. The encircling movement is reminiscent of standard late anaphase/early telophase rearrangements when the chromosomes of each sister chromosome group change position and become arranged in a semicircle before formation of the telophase nucleus (cf. Fig. 13 *b* and *l*).

Both configurations of a monocentric spindle (i.e., monopolar or star configuration) may transform into a bipolar spindle (Figs. 1 and 7), followed by a standard anaphase. The latter occurs in ~85% of the cells at 26°C. Formation of restitution nuclei (4n) usually results from the star configuration; their formation, however, is rare (<5%). Occasionally, double-chromatid chromosomes split before the formation of the restitution nucleus but do not migrate. The frequency of this process is unknown. In either case, mitosis is prolonged by a factor of 2–3. The complete dissociation of asters that leads to the formation of a monopolar spindle occurs in <5% of the cells. In contrast, a delay of the separation and migration of centrosomes (centrioles) is common and occurs in 20–50% of the cells in some batches of our cultures (~50% in Table I). In such cells, within minutes after the breaking of the nuclear envelope, all the chromosomes of the diploid set become monopolar oriented toward one center, the size of which is perhaps determined by the distance between the centrioles (9).

Monocentric spindles usually show pronounced mobility (Fig. 6). They migrate toward the cell periphery, often rotating and moving in the opposite direction; their rate of migration is comparable to, though somewhat slower than, the rate of anaphase half-spindles. The mobility of a star is usually less pronounced than that of the monopolar spindle, and its migration gradually ceases before the formation of the restitution nucleus.

Other characteristic features of monopolar spindles are the oscillatory movements discussed in the next section and their tendency to change from a monopolar to a bipolar orientation. In a few cases, however, the bipolar oriented chromosomes (in one cell, 20 out of 21) changed orientation to become monopolar, and restitution nuclei were finally formed. Such observations are rather unexpected, since it seems well-documented (4, 39) that once a bipolar kinetochore fiber is established it has considerable stability.

Oscillatory Movements of Monopolar-oriented Chromosomes

All double-chromatid chromosomes of stationary or migrating monocentric spindles execute repeated oscillatory movements toward and away from the centrosome, or a region 2–3 μm in diameter lying at the aster's center. These movements occur with kinetochores pointing to the center (Fig. 8). Occasionally, one or a few chromosomes swing around the center through an angle as great as 45° during 30 min. There is a little variation in the behavior of chromosomes from different cells with respect to paths, amplitude, or frequency of oscillations (Figs. 9, 10, 15, and Table II). Changes of direction and velocity can occur either gradually or abruptly. The shapes and slopes of the distance vs. time curve during long movements (few micrometers) towards and away from the center are often identical. They resemble mirror reflections of one another. Abrupt 180° changes in direction may occur within 10 s and often do not retrace the same paths. Due to technical limitations (see Materials and Methods) it was not possible to establish whether these movements over a longer distance occur in a "perfect" straight line with a constant velocity or are oscillatory. The most common sorts of movements are small jerks with an amplitude of 0.3–1.5 μm, during which kinetochores and adjacent parts of chromosome arms are often stretched. Consequently, the ends of chromosome arms either can follow the kinetochores synchronously or their movements may be somewhat delayed (Fig. 9).

During movement away from the center the ends of chro-

mosome arms either oscillate slightly or occasionally bend, especially if they meet some mechanical resistance (Fig. 10). The importance of this observation is stressed in the Discussion. The similarity of the movements of neighboring chromosomes varies from none at all to an almost perfect synchrony (Figs. 9 and 10) that may last as long as 45 min. The factor that seems to be a prerequisite for synchronous, or coupled, movement is the closeness of neighboring kinetochores. During the projection of a film, it is possible to see that the start of synchronous movements coincides with the lateral approach of neighboring chromosomes. Thus, the synchrony of movement is better seen during projection than in graphs that do not simultaneously show both the position of multiple kinetochores in relation to one another and their distances from the centrosome. Synchrony can most easily be traced in flat cells where chromosomes are arranged in one layer. No such correlations could be detected in rounded cells. Some other characteristics of movement are given in Fig. 10 and Table II.

TABLE II

Oscillations of Monopolar Oriented Chromosomes with Amplitude 1 μm and Higher at 21–22°C

	Single oscillation				
	Average		Velocity		Average no. of oscillations per chromosome in 10 min
	Amplitude	Duration	Average	Maximum	
	μm	min	μm/min		
Standard prometaphase	2.2	2.0	1.1	2.8	3.4
Monopolar spindle	1.8	2.1	0.9	2.3	3.4
Anaphase	1.5	1.7	0.9	2.2	3.3

Data based on Fig. 15. For comparison, the average velocity of a single chromosome group in standard anaphase is 1.3 μm/min and of migration of monopolar spindle 1.0 μm/min, the latter average of four cells. Velocities of the order 0.5–1.2 μm/min are most common in all stages for all types of movements, including spindle elongation and shortening, and those >3 μm/min are rare. The average velocities of oscillations in a single cell vary from 0.7 to 1.4 μm/min. The estimated number of oscillations per chromosome set/h, varies in different cells from 350 to 520 and is similar in all stages of mitosis.

The oscillatory movements become less pronounced in the late development of the star configuration and gradually cease during the formation of the restitution nucleus. In one of the monopolar spindles studied, the number of oscillations varied from several to 92 per single chromosome during a period of ~5 h, after which the spindle became bipolar.

Oscillations with an amplitude of 1 μm and higher are discussed below. During these oscillations, the average distance from the kinetochores to the centrosome (i.e., the radius or length of the monopolar spindle) decreases at the rate of ~1 μm/h, which is 300–500 times slower than the velocity of the oscillations. The oscillations executed by centrophilic chromosomes in standard prometaphase were indistinguishable

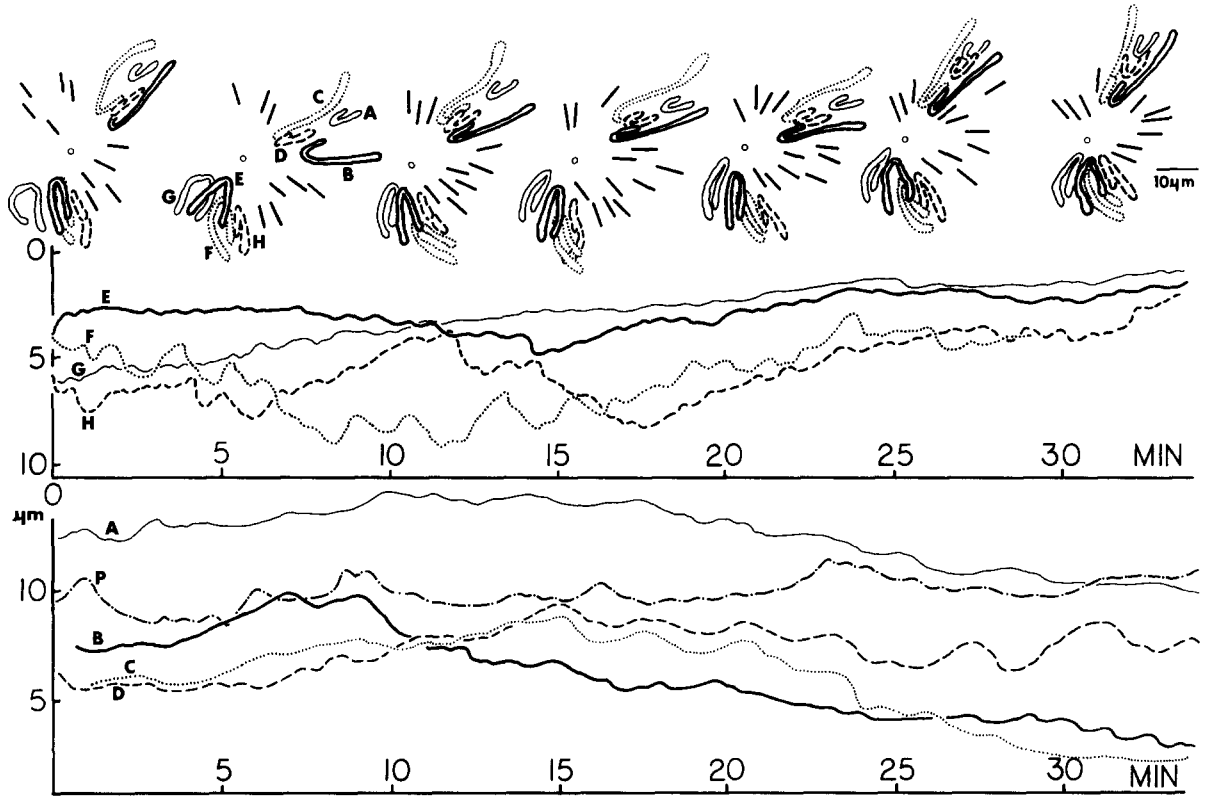


FIGURE 9 Chromosome oscillations in a monopolar spindle. Micrographs of this cell are in Fig. 7 and the paths of some chromosomes in Fig. 8. The distance from the stationary center (circle) plotted against time with a chart recorder. The center is, however, migrating slowly in the southeast direction. This is reflected in the slopes of the curves after 15 min. The position of arms of most chromosomes not analyzed is marked by short rods. Chromosomes at the edge of the spindle (*G* and *E*) and those close to the center show less pronounced oscillations. There are long periods (10–23 min for chromosomes *C* and *D*) when neighboring chromosomes oscillate in perfect synchrony. During this period their kinetochores are close together. Particle *P* oscillating between arms of chromosome *B* does not show any correlation with the movements of any chromosome. This graph demonstrates some transitory synchrony and variability in behavior between different kinetochores. Cell 88/80. 21°C.

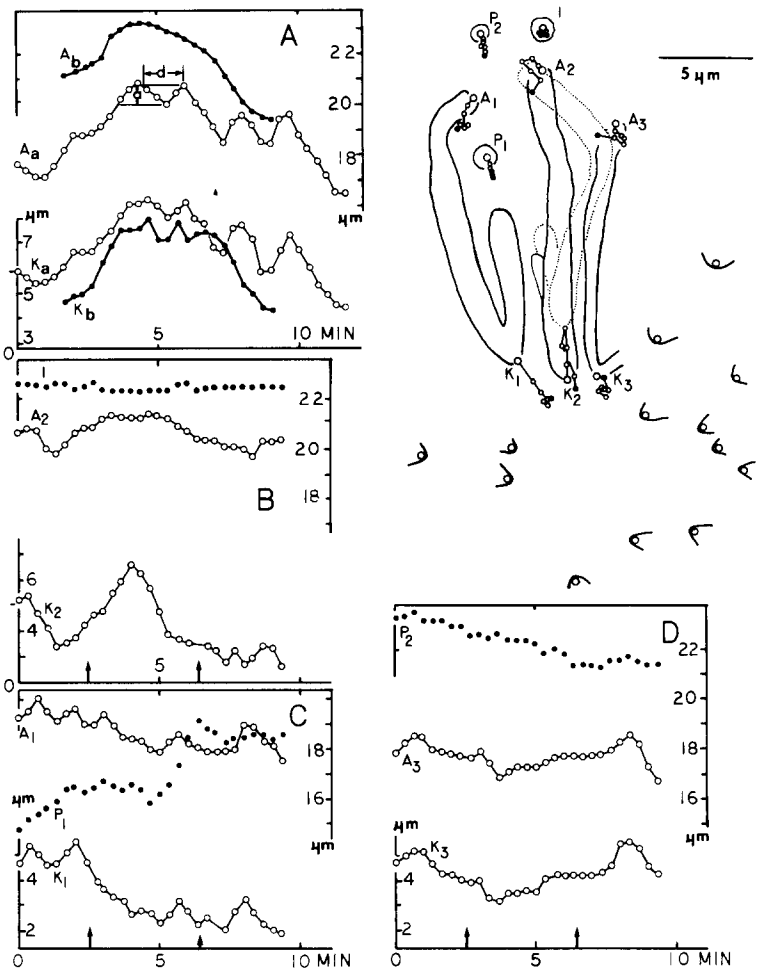


FIGURE 10 Chromosome oscillations in a monopolar spindle. The same cell as in Figs. 7–9. Amplitude (*a*) and duration (*d*) marked in *A*. The distance of kinetochores (*K*), chromosome arms (*A*), some particles (*P*) and inclusion (*I*) plotted against time. The paths of kinetochores, arm ends, etc., are drawn during the time marked by arrows in *B–D*. Measurements on the drawing are every 40 s, and on the graphs, every 20 s. *A* shows two typical behaviors of chromosomes during oscillations: perfect synchrony between kinetochores and chromosome ends (K_a–A_a) and its lack due to repeated fast oscillations resulting in slight stretching of the kinetochore region with adjacent portion of chromosome arm (K_a–A_b). *B* shows the bending of the chromosome arm (dotted outline shows this chromosome on the drawing during maximum bend) when the arm meets stationary inclusion (*I*) of unknown origin. The particles (*P*) show different mobility and the behavior of chromosomes in *C* and *D* is similar to that of those in *A*.

from those in the monocentric spindle (Figs. 11 and 12). The movements of the centrophilic chromosomes become more complex as they reorient before the start of anaphase (38). Single centrophilic chromosomes can approach the centrosome within 0.8 μm, i.e., a distance shorter than that seen at the end of anaphase. These chromosomes in asymmetric spindles having two half-spindles of different lengths, as well as those located in the astral region facing the cell periphery (Fig. 11), are often nearly motionless or move in synchrony with the movement of the centrosome (Figs. 11 and 12). During such migration, their distance from the centrosome may not change for several minutes. In contrast, multiple centrophilic chromosomes close to each other usually show pronounced motility. Similarly, chromosomes located at the periphery of the monopolar spindle (Fig. 9) usually oscillate less than those in the center among other chromosomes.

Chromosome Oscillations during Anaphase

Anaphase (single-chromatid) chromosomes are connected to one pole and are therefore monopolar oriented; an anaphase half-spindle is basically a monopolar spindle. Oscillations in anaphase half-spindles are not as pronounced as those in prometaphase but show the same characteristics (Figs. 13 and 14). The main difference between the two is the life-span of the kinetochore fiber. In the monocentric spindle or anaphase-like prometaphase the fiber lasts 10–20 times longer than in anaphase. Oscillations were detected during either bipolar or multipolar anaphase in at least 80% of the cells examined. Oscillations occur during nearly the whole period of migration, with their amplitude gradually decreasing. It should be stressed that these movements take place in the period of anaphase during which ends of the two sister chromosome groups are no longer superimposed and are thus not in close contact with each other (Fig. 13 *g–k*). This excludes any temporary stickiness between chromosome groups moving in opposite directions as a factor in the bidirectional movement. Light microscope studies do not exclude, however, connections by interzonal microtubules. The number of oscillations per chromosome during anaphase did not exceed five per chromosome; amplitudes in excess of 3 μm were observed only a few times. These observations demonstrate that anaphase movements are not perfectly synchronized and that there is considerable independence between different chromosomes of the same group. The movements are clearly visible during film projections, but, due to the crowding of the chromosomes, the kinetochores are not traceable during the backward phase of the movement in most of our film records.

DISCUSSION

Monocentric Spindle vs. Half-spindle

The monocentric spindle and the transitory configurations between monopolar and bipolar spindles are known in literature (53, 60). They are a normal feature of differentiation and meiosis in certain organisms (17, 19, 20, 34, 35, 54) and may be induced by a variety of experimental treatments in astral mitosis (18, 43) and meiosis (50). The latter report (50) is the first one in which chromosome oscillations in the monopolar

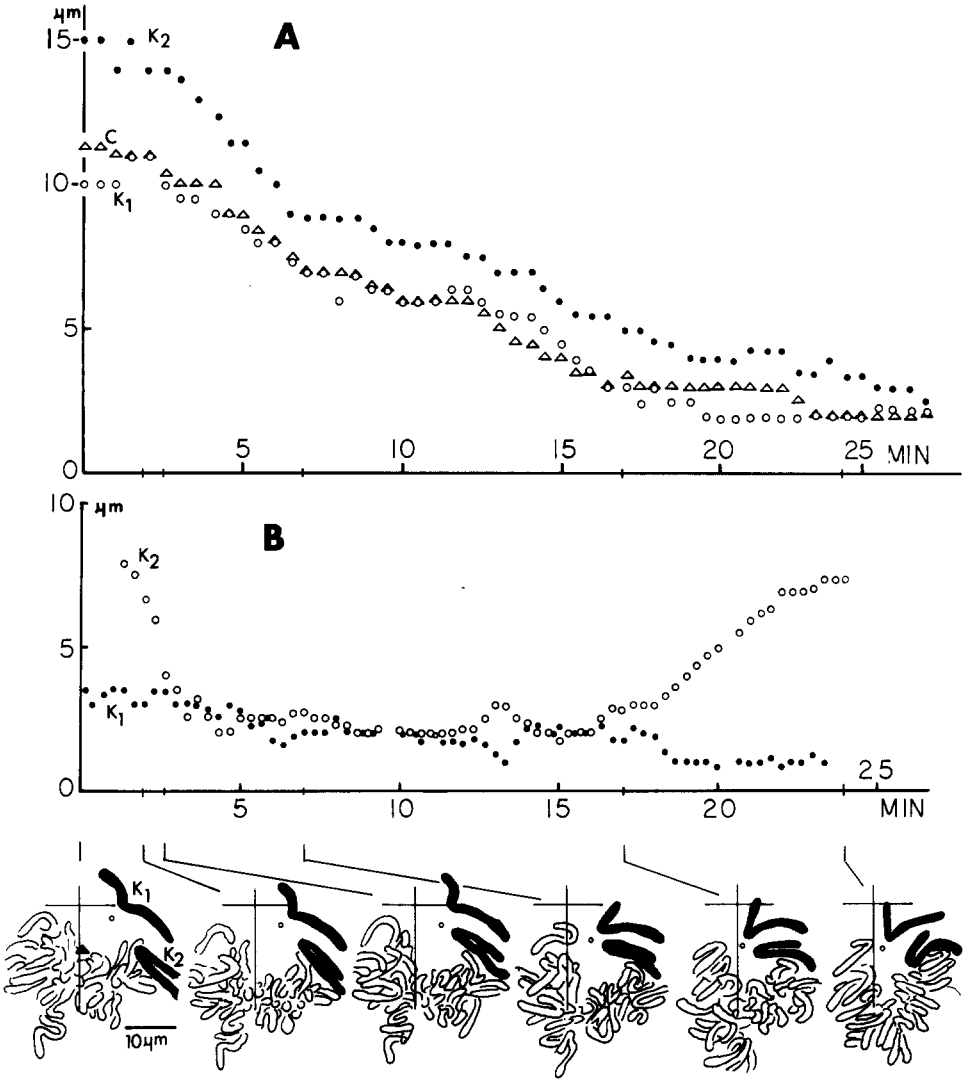
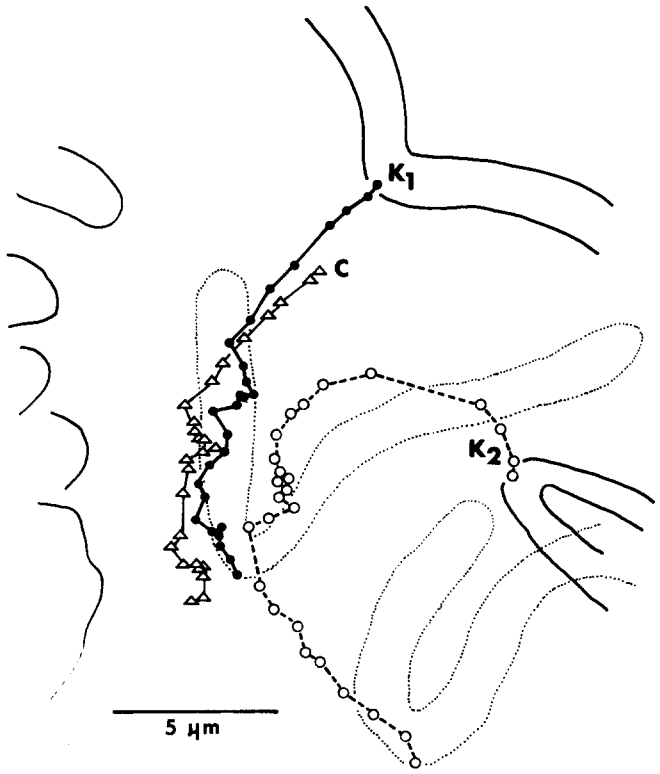


FIGURE 11 Movements of centrosome and centrophilic chromosomes in bipolar spindle. (*A*) The distance of kinetochores (K_1 and K_2) and centrosome (*C*) from an arbitrary reference point (black triangle in the drawing at 0 time) and (*B*) the distance of the same kinetochores from the centrosome (small circle in the drawing), plotted against time. Cross marks the same position in the frame. The paths of these chromosome kinetochores are shown in Fig. 12. The curves in *A* and *B* demonstrate that the kinetochore may follow very closely the migration of the centrosome when the distance and presumably the length of the kinetochore fiber do not change during a comparatively long migration, e.g., K_1 in 0–7 min. The centrosome migrates in steps interrupted by stationary periods. Kinetochore K_2 reorients at ~17 min and returns to the plate, whereas K_1 reorients only at the time of 65 min. K_1 is an example of a single chromosome located between the centrosome and the cell periphery. Such chromosomes are usually nearly stationary with respect to the centrosome (cf. text and Fig. 9). Cell 58/80. 21°C.



meiotic spindle were described in living cells. The present report demonstrates, however, that a monocentric mitotic spindle may be a common transitory phase of normal prometaphase in primary tissue cultures of newt lung epithelium and that the behavior of centrophilic (38, 59, 63) chromosomes is the same in standard prometaphase as in monopolar spindles. The monocentric spindle discussed here is, therefore, an extreme case of a centrophilic arrangement with all the chromosomes persistently centrophilic (monopolar). Such spindles, however, retain for a long time the capability to become bipolar.

A monopolar prometaphase spindle is, in principle, half of a bipolar spindle and is similar, in many respects, to a standard anaphase half-spindle. Both can migrate long distances and support chromosome movements towards and away from the pole. The difference is in the chromosome structure (double- vs. single-chromatid) and in the duration of these configura-

FIGURE 12 Paths of kinetochores (*K*) and centrosome (*C*). The displacements are marked every minute. The movements of these chromosomes and centrosome are shown in Fig. 11. The centrosome (*C*) migrates basically along straight lines interrupted by periods of irregular rocking movements. Kinetochores follow closely its behavior either all the time (K_1) or only part of the time (K_2). Chromosome outlines are drawn by continuous line at the start of measurements and dotted at the end. This cell formed normal metaphase followed by anaphase and cleavage. Cell 58/80. 21°C.

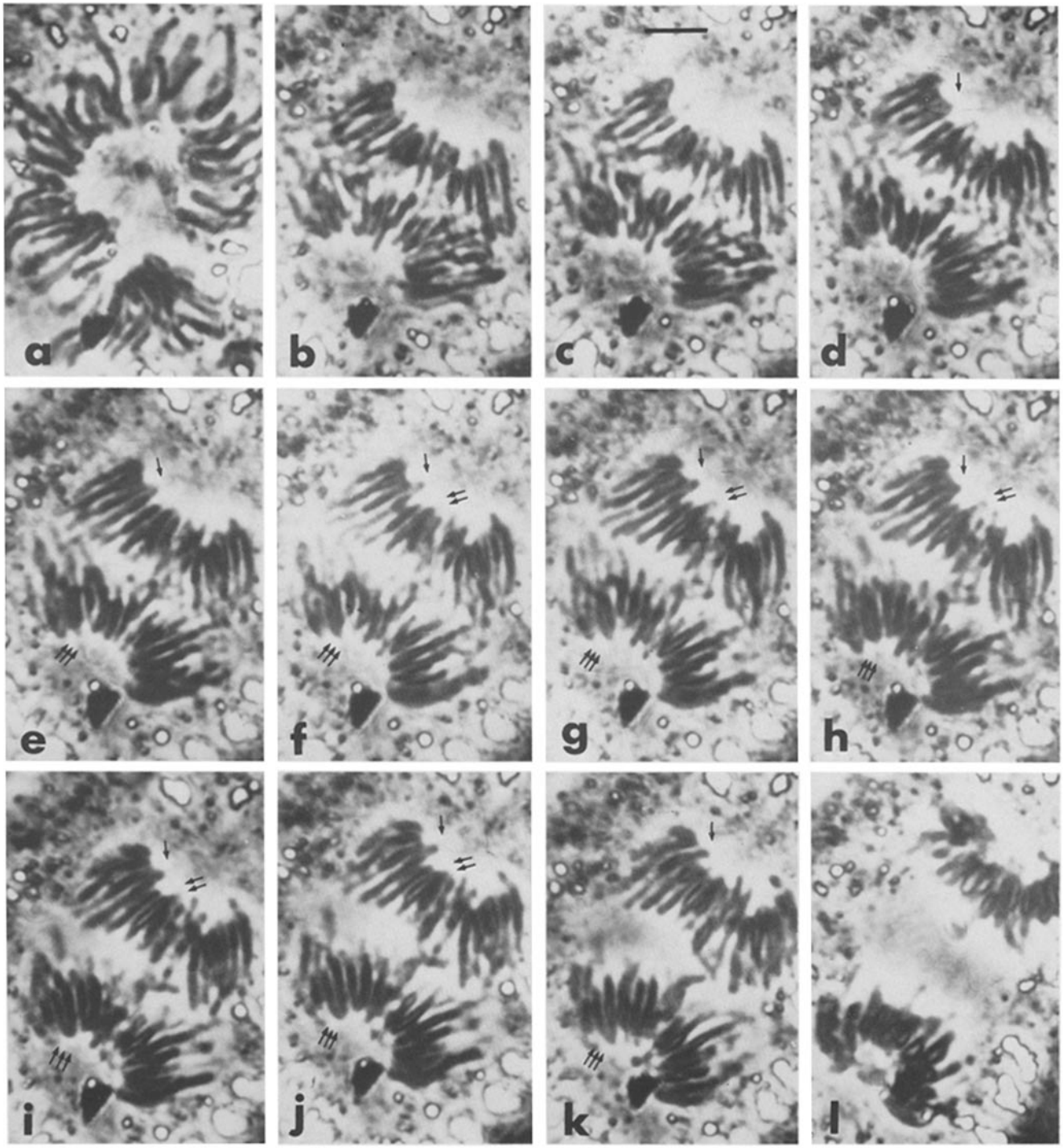


FIGURE 13 Oscillations of chromosomes in anaphase. Arrows point to particular region where chromosome oscillations can be traced on micrographs. All chromosomes execute such oscillations, but they are usually most clearly seen at the edges of chromosome group. The course of some oscillations is shown in Fig. 14. Time in minutes and seconds after *a*: *b*, 95; *c*, 95.30; *d*, 97; *e*, 97.30; *f*, 98; *g*, 98.30; *h*, 99; *i*, 99.30; *j*, 100; *k*, 102; *l*, 115. Cell 79/80. 21°C. Bar in *c*, 10 μm.

tions. The monocentric prometaphase spindle may support oscillations 20 times longer than the duration of anaphase.

Oscillations of Monopolar-oriented Chromosomes

Two striking features of a monopolar spindle are the continuous chromosome oscillations towards and away from its single pole and its tendency for migration towards the cell periphery. Oscillations of monopolar-oriented chromosomes may be a consequence of attachment to the single pole. This has been observed not only in tissue cultures (7, 9, 38, 42, 46) but also in diatoms (58). Oscillations in the newt epithelium occur in all stages of standard mitosis including anaphase, where they have been known for a long time (14) although seldom reported (45). The striking feature of oscillations in prometaphase is that while the average distance from the pole corresponds to that in early or mid-anaphase, some chromosomes may temporarily move even closer to the pole than at the end of anaphase, though they will soon move away from the pole to

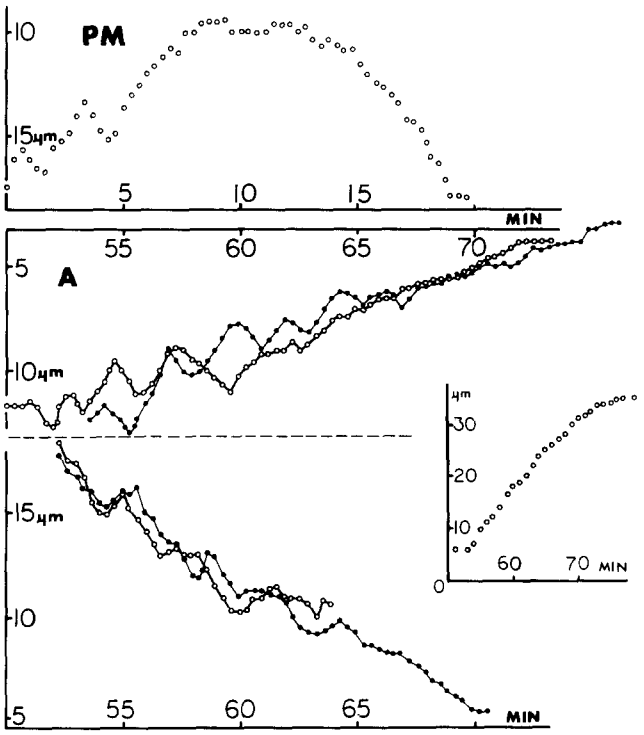


FIGURE 14 Oscillations of chromosomes in prometaphase (*PM*) and anaphase (*A*). The distance from the stationary center at each pole (approximate position of the centrosome at the start of anaphase plotted against time for two neighboring chromosomes). The upper and lower curves are not for sister chromosomes. Elongation of the poles is not shown on this graph. (*Inset*) The distance between two sister chromosome groups plotted against time. Prometaphase oscillations show the same characteristics as in anaphase (time 0–5 and 52–63). Chromosome in prometaphase moves toward the pole where it oscillates slightly for ~5 min and then reorients and migrates back to the plate. Lower curve in anaphase shows that there are transitory periods when neighboring chromosomes move synchronously. Micrographs of this cell are in Fig. 13. Cell 79/80. 21°C.

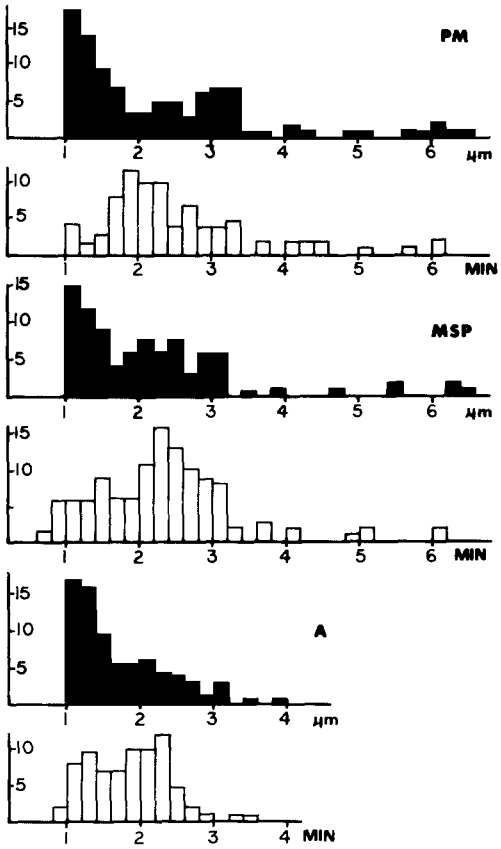


FIGURE 15 Distribution of oscillations in prometaphase (*PM*), monopolar spindle (*MSP*) and anaphase (*A*). The number of oscillations (vertical axis) plotted as a function of the amplitude (black blocks) and duration (white blocks). See Fig. 10 *A* for explanation of amplitude and direction. Only oscillations with amplitude of 1 μm and higher were measured. The number of oscillations with 1-μ amplitude is the highest, indicating increasing number of oscillations with much lower amplitude. There is a slight peak of longer oscillations both in prometaphase and in the monopolar spindle, less evident in anaphase. The amplitude and duration of oscillations are practically the same in prometaphase and monopolar spindles and decrease in anaphase. This histogram was plotted partly from the measurements made directly on the graphs and partly during frame-by-frame film projection. The numbers of oscillations measured were as follows: for PM, MSP, and A, amplitudes 102, 102, and 72, and durations 107, 121, and 74, respectively. The measurements of oscillations in monopolar spindle and anaphase were done on cells where at least 11 chromosomes could be followed in one chromosome group.

which they point. Thus, the chromosomal (kinetochore) fiber does not irreversibly disassemble during prometaphase. Its ability to support oscillation vanishes rapidly, however, during the progress of anaphase. The present analysis does not detect any basic differences between chromosome oscillations in any stages of mitosis (Fig. 15). The fact that the duration and amplitude of single oscillations gradually decrease during anaphase is to be expected, because the spindle disassembles irreversibly at this time. The disappearance of oscillations is especially striking during the formation of the restitution nucleus when they decrease as rapidly as in later stages of standard anaphase.

These observations raise questions concerning chromosome transport and the regulatory factors influencing spindle function. The major question is: Why instead of a single migration to the pole do monopolar-oriented chromosomes oscillate in all stages of mitosis? This fundamental question can, at the moment, be given only very speculative answers involving the process of microtubule nucleation, kinetochore activity, and the regulation of an unknown molecular basis essential to chromosome movement. The discussion of these points is beyond the scope of the present paper, but some aspects are mentioned elsewhere (6, 7, 8). The avoidance of such discussion is also prompted by preliminary efforts to determine whether there is any functional relation between neighboring kinetochore fibers. The data provided do not present clear-cut evidence. One is left with the impression of a comparatively random distribution of oscillations with localized domains in the spindle in which oscillations are in perfect synchrony. These preliminary data are most easily explained by lateral associations (interactions?) between neighboring kinetochore fibers (5, 6).

"Pull-push" Mechanism?

There are two phases in a single oscillatory motion: a shortening and an increasing of kinetochore-to-pole distance. EM data (9, 38, 40) indicate that kinetochores are connected to the poles by straight fibers that end at the kinetochores.

Therefore, during an oscillation the kinetochore fibers both

shorten and elongate. This fact, combined with the absence of the central spindle fibers from the monocentric spindles, eliminates the possibility of sliding along central spindle fibers as proposed for the bipolar spindle in grasshopper meiosis by Bělař (12) and, more recently, by Tippit et al. (58). Central spindle does not exist, however, either in spontaneous monocentric spindles in the newt lung epithelium described here or in those experimentally produced in sea urchin eggs by Paweletz and Mazia (40) and Mazia et al. (33).

The fact that the kinetochore and proximal regions of the chromosome arms are often stretched during a migration toward a pole suggests that the motion results from a pulling action by the chromosomal fibers. The backward chromosome motion in the newt monocentric spindle does not result in kinetochore deformation. The bending of chromosome arms, however, is taken as evidence of "pushing" by the chromosomal fibers. It represents one of the few clear examples of such activity observed in living cells.[1] Such bending of chromosome arms has also been reported by Metz (35, 36) in *Sciara* and by Scott (56) in *Micromalthus* and explained by these authors as pushing due to an autonomous chromosome migration. Single movements in the newt last too long and cover distances too great to be explained by elastic deformation of the spindle matrix (44) or the recoil of the chromosomes stretched during their poleward phase. The possibility of the existence of another kinetochore fiber pointing away from the pole, as in the cases described by Huth from the drawings of Bělař (13), has also been excluded (9, 38, 40; J. Molè-Bajer and A. S. Bajer, manuscript submitted for publication).

This leaves the possibility that the elongation of the monopolar kinetochore fiber pushes the chromosomes. "Pushing" by microtubules (spindle-fiber microtubules) has been reported during the breaking of the nuclear envelope (4) and the formation of kinetochore fibers (24). Schrader (53) reviews several examples on fixed material which are best explained by pushing due to the elongation of chromosomal fibers. Experiments on astral spindles (10) shift the equilibrium between tubulin-microtubules toward disassembly, but still permit chromosomal movements towards the pole. In these experiments, backward movements cease during an initial phase of low temperature shock in all stages of mitosis; kinetochore microtubules still exist, while nonkinetochore microtubules disassemble. Conversely, a shift toward assembly might result in a "push" of chromosomes within the astral spindle (23, 48).

Does assembly, which might result in backward "push," occur in anaphase? The formation of new microtubules has been documented in anaphase of anastral spindle of *Haemanthus* endosperm (8; J. De Mey et al. *Proc. Natl. Acad. Sci. U. S. A.*, Vol. 79, in press); the importance of anaphase assembly has been pointed out by Schmit-Benner and Lambert (52). It must be stressed, however, that straightforward evidence of the relation between assembly and pushing by single kinetochore fibers in anastral spindle does not exist (see Note Added in Proof). If these observations (8, 10, 19, 23, 48) also apply to anaphase, then chromosome transport in all stages of mitosis may involve a "pull/push" principle in both astral and anastral mitosis of higher organisms.

The direction of chromosome movements in the astral spindle may be determained by a subtle relationship between at least three factors: the interaction between various types of spindle fibers (5) (kinetochore/nonkinetochore microtubules), their rates of assembly-disassembly (22, 50), and the manner of kinetochore fiber anchorage. Whatever the mechanism of backward movement may be, the kinetochore fibers change length repeatedly for a long time during prometaphase/metaphase when chromosomes are double chromatid and for a short time in anaphase when chromosomes are single chromatid.

The occasionally observed synchronous oscillatory movement of neighboring chromosomes is considered as evidence that one chromosome may influence the behavior of its neighbor. Therefore, each kinetochore fiber may serve, to a variable extent, as a mechanical support for the other fibers. The synchronous migration of a kinetochore with the centrosome when there is a very small distance between them (1 μm) is considered as evidence that a kinetochore fiber can be transiently but firmly anchored at the centrosomal region in both prometaphase and anaphase.

Autonomy of the Half-spindle and the Function of the Central Spindle

The autonomy of half-spindles is most likely another important and general feature of mitosis that has gained additional experimental support through studies on sea urchin eggs (33, 40, 57). Regardless of whether the migration of the half-spindle in standard anaphase and migration of prometaphase half-spindle in anaphaselike prometaphase are analogous, the present data demonstrate that two sister prometaphase half-spindles with monopolar-oriented metaphase chromosomes can migrate over distances longer than anaphase separation. This separation occurs without any microtubules in the false interzone in both the newt (J. Molè-Bajer and A. S. Bajer, manuscript submitted for publication) and PtK (3, 15) cells. If these observations apply to other standard astral mitosis, they rule out an "active" role of the interzone in the spindle elongation during standard astral anaphase, as suggested by the most recent sliding hypothesis (Margolis et al [31]). On the contrary, the presence of microtubules in the interzone slows down chromosome movements in *Haemanthus* endosperm (37), newt (7), and PtK_1 (25) cells.

The mechanism of aster migration and the role of kinetochores (2, 29) and centrioles in this process are unknown and invite several speculations (1, 3, 16). Aster migration is well-documented, e.g., during insect development (51, 61, 62). The elongation of the spindle in prometaphase was described for PtK_1 cells (46) and crane fly spermatocytes (27) and is probably caused by aster migration. This process is, however, potentially disruptive during prometaphase. Anaphaselike prometaphase is a rare event and is prevented by the formation of bipolar kinetochore fibers. It is evident, therefore, that, during standard prometaphase, bipolar chromosomal fibers form the spindle, that pulls and, consequently, holds asters together. The existence of a central spindle is well-documented in several lower organisms (21, 26) but, as stressed by Mazia (32), an equivalent structure has not been demonstrated in astral mitosis and is not needed to explain the standard mitotic events.

It is a pleasure to thank Marc Stone and Suzanne Paulaitis for expert assistance with tissue culture, and Chris Cypher, Drs. Roland Dietz, and Patricia Harris for stimulating discussions. My thanks are extended also to the JCB reviewers, particularly Dr. J. R. McIntosh, whose constructive and detailed comments helped to present the data in a more cogent form. Thanks also to Anna-Maria Bajer, who helped to simplify the style of this presentation.

[1] Very slow continuous backward movements have been documented by Dietz in the meiosis of crane fly spermatocytes. (R. Dietz, 1956, Habil. Thesis. University of Tübingen, Germany).

This work was supported by the National Institute of Health grants GM 21741 and GM 26121.

Received for publication 8 April 1981, and in revised form 6 November 1981.

Note Added in Proof: Rapid polymerization of polar (nonkinetochore) MTs caused by taxol in anaphase of *Haemanthus* endosperm (Bajer and Molè-Bajer, manuscript submitted for publication) often results in the stretching and breaking of trailing chromosome arms. Broken fragments are transported away from the pole, often "riding on the tips" of elongating MTs. This provides direct evidence for "push" by rapidly elongating MTs in higher plant anaphase, under these experimental conditions.

REFERENCES

1. Albrecht-Buehler, G. 1981. Does the geometric design of centrioles imply their function? *Cell Motility.* 1:237–247.
2. Alov, I. A., and S. L. Lyubskii. 1974. Experimental study of the functional morphology of the kinetochore in mitosis. *Byul. 'Éksper. Biol. Med.* 78:91–94.
3. Aubin, J. E., M. Osborn, and K. Weber. 1980. Variation in the distribution and migration of centriole duplexes in mitotic PtK_2 cells studied by immunofluorescence microscopy. *J. Cell Sci.* 43:177–194.
4. Bajer, A. S., and J. Molè-Bajer. 1972. Spindle dynamics and chromosome movements. *Int. Rev. Cytol.* 34(Suppl. 3):1–271.
5. Bajer, A., and J. Molè-Bajer. 1975. Lateral movements in the spindle and the mechanism of mitosis. *In* Molecules and Cell Movement. S. Inoué and R. E. Stevens, editors. Raven Press, N. Y. 77–96.
6. Bajer, A., and J. Molè-Bajer. 1979. Anaphase—Stage Unknown. *In* Cell Motility: Molecules and Organization. S. Hatano, H. Ishikawa, and H. Sato, editors. Univ. of Tokyo Press. 569–591.
7. Bajer, A. S., and J. Molè-Bajer. 1982. Mitosis: observations on living cells lead to new concepts. *In* Mitotic Cycle. A. M. Zimmerman and A. Forer, editors. Academic Press, Inc., N.Y. 277–299.
8. Bajer, A. S., and J. Molè-Bajer. Asters, poles and transport properties within spindle-like microtubule arrays. *Cold Spring Harbor Symp. Quant. Biol.* 46:263–283.
9. Bajer, A. S., J. Molè-Bajer, M. De Brabander, J. De Mey, G. Geuens, and S. Paulaitis. 1980. Aster migration and functional autonomy of monopolar spindle. *In* Microtubules and Microtubule Inhibitors. II. M. De Brabander and J. De Mey, editors. Elsevier, N.Y. 399–425.
10. Bajer, A. S., J. Molè-Bajer, and M. Stone. 1980. Non-kinetochore microtubules determine pattern of chromosome migration. J. Cell Biol. 87(2, Pt. 2):238*a* (Abstr.).
11. Barber, H., and H. G. Callan. 1943. The effects of cold and colchicine on mitosis in the newt. *Proc. R. Soc. Lond. B. Ser. Biol.* 131:258–270.
12. Bělař, K. 1927. Beiträge zur Kenntnis des Mechanismus der indirecten Kernteilung. *Naturwiss.* 15:725–734.
13. Bělař, K., and W. Huth. 1933. Zur Teilungsautonomie der Chromosomen. *Z. Zellforsch. Mikrosk. Anat.* 17:51–66.
14. Bloom, W., and R. E. Zirkle. About 1954 (film not dated). Mitosis in The Newt Cells. 16mm film. University of Chicago Bookstore. Chicago, Ill. Zirkle (63) mentions that sequences in this film were recorded before 1955.
15. Brenner, S., A. Branch, S. Meredith, and M. W. Berns. 1977. Absence of centrioles from spindle poles of rat kangaroo (PtK_2) cells undergoing meioticlike reduction division in vitro. *J. Cell Biol.* 72:368–379.
16. Bornens, M. 1979. The centriole as a gyroscopic oscillator. *Biol. Cell.* 35:115–132.
17. Camenzind, R., and T. Fux. 1977. Dynamics and ultrastructure of monocentric chromosome movement. *Caryologia.* 130(2):127–150.
18. Cox, M. S., P. N. Rao, and R. B. Brinkley. 1979. Action of nitrous oxide and griseofulvin on microtubules and chromosome movements in dividing cells. *In* Effects of Drugs on the Cell Nucleus. Bush, H., S. T. Crooke, and Y. Doskal, editors. Academic Press, Inc., N.Y. 1:521–549.
19. Fankhauser, G. 1934. Cytological studies on egg fragments of the salamander Triton. *J. Exp. Zool.* 67:159–215.
20. Fux, T. 1976. The kinetochores of amphitelic univalents in a functionally monocentric spindle. *Microbios Lett.* 1:109–115.
21. Heath, I. B. 1980. Variant mitoses in lower eukaryotes: indicators of the evolution of mitosis? *Int. Rev. Cytol.* 64:1–80.
22. Inoué, S., J. Fuseler, E. D. Salmon, and G. W. Ellis. 1975. Functional organization of mitotic microtubules. *Biophys. J.* 15:725–744.
23. Inoué, S., and H. Sato. 1967. Cell motility by labile association of molecules: the nature of mitotic spindle fibers and their role in chromosome movement. *J. Gen. Physiol.* 50:259–292.
24. Izutsu, K., H. Sato, H. Hakabyashi, and N. Aoki. 1977. The behavior of spindle fibers and movement of chromosomes in dividing grasshopper spermatocytes. *Cell Struct. Funct.* 2: 119–133.
25. Kronebush, P. J., and G. G. Borisy. 1981. Anaphase pole movement after central spindle disruption in PtK_1 cells. *J. Cell Biol.* 91(2, Pt. 2):319*a* (Abstr.).
26. Kubai, D. F. 1975. The evolution of the mitotic spindle. *Int. Rev. Cytol.* 43:167–227.
27. La Fountain, J. R., Jr. 1972. Spindle shape changes as an indicator of force production in crane-fly spermatocytes. *J. Cell Sci.* 10:79–93.
28. Leibovitz, A. 1963. Growth and maintenance of tissue-cell cultures in free gas exchange with atmosphere. *Am. J. Hyg.* 78:173–180.
29. Lyubskii, S. L. 1974. Changes in the ultrastructure of the kinetochore during mitosis. *Byull. Eksp. Biol. Med.* 77:113–116.
30. Margolis, R. L., L. Wilson, and B. I. Kiefer. 1978. Mitotic mechanism based on intrinsic microtubule behavior. *Nature (Lond.).* 272:450–452.
31. Mazia, D. 1961. Mitosis and physiology of cell division. *In* The Cell. J. Brachet and A. E. Mirsky, editors. Academic Press, Inc., N.Y. 3:77–412.
32. Mazia, D. 1978. Origin of twoness in cell reproduction. *In* ICN-UCLA Symposium on Molecular and Cellular Biology. E. R. Dirksen, D. M. Prescott, and C. F. Fox, editors. Academic Press, Inc., New York 12:1–14.
33. Mazia, D., N. Paweletz, G. Sluder, and E. M. Finze. 1981. Cooperation of kinetochores and the pole in the establishment of monopolar mitotic apparatus. *Proc. Natl. Acad. Sci. U. S. A.* 78:377–381.
34. Metz, C. W. 1933. Monocentric mitosis with segregation of chromosomes in *Sciara* and its bearing on the mechanism of mitosis. *Biol. Bull.* 64:333–347.
35. Metz, C. W. 1936. Factors influencing chromosome movements in mitosis. Cytologia. 7: 219–231.
36. Molè-Bajer, J. 1975. The role of centrioles in the development of the astral spindle (newt). *Cytobios.* 13:117–140.
37. Molè-Bajer, J., and A. Bajer. 1978. The effect of delayed microtubule disassembly on chromosome movement. *J. Cell Biol.* 79(2, Pt. 2):283*a* (Abstr.).
38. Molè-Bajer, J., A. Bajer, and A. Owczarzak. 1975. Chromosome movements in prometaphase and aster transport in the newt. *Cytobios.* 13:45–65.
39. Nicklas, R. B. 1971. Mitosis. *In* Advances in Cell Biology. Prescott, D. M., L. Goldstein, and E. H. McConkey, editors. Appleton, N.Y. 2:225–297.
40. Paweletz, N., and D. Mazia. 1979. Fine structure of the mitotic cycle of unfertilized sea urchin eggs activated by ammoniacal water. *Eur. J. Cell Biol.* 20:37–44.
41. Peters, J. J. 1946. A cytological study of mitosis in the cornea of *Triturus viridescens* during recovery after colchicine treatment. *J. Exp. Zool.* 103:33–59.
42. Petzelt, C. 1979. Biochemistry of the mitotic spindle. *Int. Rev. Cytol.* 60:53–92.
43. Rao, P. N., and J. Engelberg. 1966. Mitotic non-disjunction sister chronatids and anomalous mitosis induced by low temperatures in HeLa cells. *Exp. Cell Res.* 43:332–342.
44. Rattner, J. B., and M. W. Berns. 1976. Distribution of microtubules during centriole separation in rat kangaroo (*Protorous*) cells. *Cytobios.* 15:37–43.
45. Rieder, C. L. 1977. An *in vitro* light and electron microscopic study of anaphase chromosome movement in normal and temperature elevated *Taricha* lung cells. PhD thesis, University of Oregon, Eugene, Oregon.
46. Roos, U. 1976. Light and electron microscopy of rat kangaroo cells in mitosis. *Chromosoma (Berl.)* 54:363–385.
47. Rose, G. G., C. M. Pomerat, T. O. Shindler, and J. B. Trunnell. 1958. A cellophane strip technique for culturing tissue in multipurpose chambers. *J. Biophys. Biochem. Cytol.* 4: 761–764.
48. Salmon, E. D. 1975. Spindle microtubules: thermodynamics of *in vivo* assembly and role in chromosome movement. *Ann. N. Y. Acad. Sci.* 253:383–406.
49. Sato, H., Y. Ohnuki, and K. Fujiwara. 1976. Immunofluorescent antitubulin staining of spindle microtubules and critique for the technique. *In* Cell Motility. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor, N.Y. 419–433.
50. Sato, H., Y. Ohnuki, and Y. Sato. 1979. Assembly and disassembly of the mitotic spindle. *In* Cell Motility: Molecules and Organization. S. Hatano, H. Ishikawa, and S. Sato, editors. Yamada Conference I. University of Tokyo Press. 551–568.
51. Sauer, H. W. 1966. Zeitraffer-mikro-film-analyse embryonaler Differenzierungsphasen von *Gryllus domesticus. Z. Morphol. Ökol. Tiere.* 56:143–251.
52. Schmit-Benner, A. C., and A. M. Lambert. 1981. Immunofluorescent antitubulin staining of plant cells during mitosis under control and colchicine, vinblastine treatments. *Biol. Cell.* 41:24a (Abstr.).
53. Schrader, F. 1953. Mitosis. Columbia University Press, N.Y.
54. Scott, A. C. 1936. Haploidy and aberrant spermatogenesis in a coleopteran, Micromalthus debilis Le Contre. J. Morphol. 59:485–515.
55. Sentein, P. 1979. Nocodazole, an antitubulin substance, whose action is different from that of colchicine. Effects on segmentation mitoses. I. Light Microscopy. *Arch. Biol.* 90: 459–479.
56. Seto, T., J. Kezer, and Ch. M. Pomerat. 1969. A cinematographic study of meiosis in salamander spermatocytes in vitro. *Z. Zellforsch. Mikrosk. Anat.* 94:407–424.
57. Sluder, G. 1978. The reproduction of mitotic centers: new information of an old experiment. ICN-UCLA Symposium of Molecular and Cellular Biology. E. R. Dirksen, D. M. Presscott, and C. F. Fox, editors. Academic Press, Inc., N.Y. 12:563–569.
58. Tippit, D. H., J. D. Pickett-Heaps, and R. Leslie. 1980. Cell division in two large pennate diatoms Hantzschia and Nitzschia. III. A new proposal for kinetochore function during prometaphase. *J. Cell Biol.* 86:402–416.
59. Uretz, R. B., W. Bloom, and R. E. Zirkle. 1954. Irradiation of parts of individual cells. II. *Science (Wash. D. C.).* 120:197–199.
60. Wilson, E. B. 1924. The Cell in Development and Heredity, 3rd Ed. Macmillan, N. Y.
61. Wolf, R. 1975. A new migration mechanism of cleavage nuclei based on the cytaster. *Verh. Dtsch. Zool. Ges.* 1974:174–178.
62. Wolf, R. 1978. The cytaster, a colchicine-sensitive migration organelle of cleavage nuclei in an incest egg. *Dev. Biol.* 62:464–472.
63. Zirkle, R. E. 1957. Partial cell irridation. *Adv. Biol. Med. Phys.* 5:103–146.
64. Zirkle, R. E. 1970. Ultraviolet-microbeam irradiation of newt cell cytoplasm. Spindle destruction, false anaphase, and delay of true anaphase. *Radiat. Res.* 41:516–537.